UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte PRABHAKARA V. CHOUDARY, ABIODUN A. OGUNJIMI, and JOHN M. CHANDLER

Appeal No. 2004-2134
Application No. 09/425,075

HEARD: February 10, 2005

Before SCHEINER, ADAMS and GREEN, Administrative Patent Judges.

GREEN, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 36-39 and 41-50. Claims 36 and 41 are representative of the subject matter on appeal, and read as follows:

36. A method for production of an antibody that specifically binds an antigen of interest, the method comprising the steps of:

culturing a recombinant *Pichia* cell, the cell comprising a vector comprising a first and second expression cassette, wherein:

said first expression cassette comprises a first promoter operably linked to a nucleic acid encoding an immunoglobulin light chain operably linked to a first signal peptide;

said second expression cassette comprises a second promoter operably linked to a nucleic acid encoding an immunoglobulin heavy chain operably linked to a second signal peptide,

and said culturing provides for expression of the immunoglobulin light and heavy chains; and

harvesting specific antigen-binding antibody from culture supernatant, which antibody specifically binds an antigen of interest.

41. The method of Claim 36, wherein said antibody specifically binds dioxin.

The examiner relies upon the following references:

Robinson et al. (Robinson)	6,204,023	Mar. 20, 2001
Vanderlaan et al. (Vanderlaan)	5,429,925	Jul. 04, 1995

Horwitz et al. (Horwitz) "Secretion of Functional Antibody and Fab Fragment from Yeast Cells," Proc. Natl. Acad. Sci. USA, Vol. 85, pp.8678-8682 (1988)

Cregg et al. (Cregg) "Development of the Methylotrophic Yeast, *Pichia Pastoris*, as a host System for the Production of Foreign Proteins," Developments in Industrial Microbiology, Vol. 29, pp.33-41 (1988)

The Invitrogen Catalog "Yeast Expression," pp. 14-19 (1997)

In addition, appellants rely upon the following references:

Holliger "Expression of Antibody Fragments in *Pichia pastoris*," Methods in Molecular Biology, Vol. 178, pp. 348-357 (2002)

Pennell et al. (Pennell) "In vitro Production of Recombinant Antibody Fragments in *Pichia pastoris*," Res Immunol, Vol. 149, pp. 599-603 (1998)

Claims 36-39 and 42-50[1] stand rejected under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Horwitz, Cregg, the Invitrogen Catalog and Robinson. In addition, claims 36-39 and 41-50[2] stand rejected under 35 U.S.C. § 103(a) as being obvious over the previous combination of references as further combined with Vanderlaan. After careful review of the record and consideration of the issues before us, we affirm both rejections.

BACKGROUND

The invention pertains to the production of functionally assembled antigen-specific intact monoclonal antibodies produced by the transformation of the methylotropic yeast, *Pichia pastoris*, with immunoglobulin genes. See Specification, page 1. According to the specification,

> [t]he method of the invention for production of functionally assembled antigen-specific intact monoclonal antibody, using transformation of *P. pastoris*, has a general utility and essentially any antibody can be produced or secreted by *P. pastoris* as long as the yeast expression vector carrying antibody genes can be appropriately assembled.

Id. at 6.

More specifically,

> a recombinant yeast expression vector (pPICZα) with dual expression cassettes is constructed, each cassette carrying the

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[1] The Examiner's Answer states that claims 36-40 and 42-49 stand rejected. See Examiner's Answer, page 3. The Appeal Brief, however, states that claims 36-39 and 42-50 stand rejected. See Appeal Brief, page 5. As claim 40 is not pending, the examiner's statement appears to be a typographical error, and we thus decide the appeal as it pertains to claim 36-39 and 42-50.

[2] Again, the Examiner's Answer states that the rejection is applied to claims 36-49, see Examiner's Answer, page 11, while the Appeal Brief states that it apples to claims 36-39 and 41-50. For the reasons set forth in the previous footnote, we are again treating the examiner's statement as a typographical error.

> inducible alcohol oxidase (AOX1) promoter, fused to the *Saccharomyces cerevisiae* α-factor signal sequence. *P. pastoris* is then transformed with these constructs, and the resulting transformant secretes functionally assembled intact recombinant antibody molecules into the medium from where it is readily recovered using affinity purification procedures.
>
> Specificity of the produced antibody is determined by demonstrating the antibody-specific mRNA synthesis in recombinant yeast using Northern blot analysis. When the specific antibody is produced, immunoblot and ELISA analyses of concentrated culture supernatants harvested a few days post-transformation reveal the presence of antigen-specific human, mouse or other mammalian species-specific immunoglobulins. Assaying of the culture supernatants by ELISA then shows specific binding activity to the specific antigen against which the antibody is raised or to a crossreactive congener. The binding affinity of the produced recombinant IgG is the same as, and/or comparable to, that of the parent IgG.

Id. at 6-7.

DISCUSSION

Claims 36-39 and 42-50 stand rejected under 35 U.S.C. § 103(a) as being rendered obvious by the combination of Horwitz, Cregg, the Invitrogen Catalog and Robinson. As the claims stand or fall together, see Appeal Brief, page 7, we focus our analysis on the method of claim 36.

Horwitz is cited for teaching a method "for the production of an antibody in S. cerevisiae yeast cells with the vectors comprising cDNA encoding for an antibody, a promoter and transcription terminator, and signal sequence." Examiner's Answer, page 4. According to the rejection, "Horwitz [ ] does not teach a recombinant host P. pastoris, SMD1168 transformed with a vector for

expression with dual expression cassettes, the Pichia alcohol oxidase promoter, alpha factor signal sequence, AOX1-P promoter." Id.

Cregg is cited for teaching the use of the AOX1 for the expression of foreign proteins in Pichia pastoris. See id.

Robinson is cited for teaching "methods of expression of antibodies in yeast with expression plasmids comprising the light chain and heavy chains each attached to a yeast promoter and terminator and are placed on the same plasmid." Id. Robinson is also cited for teaching that "yeast is a preferred host because yeast provides substantial advantages for the production of immunoglobulin light and heavy chains because yeast carry out post-translational peptide modifications including glycosylation," and for teaching that "a number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number plasmids which can be used for overt production of the proteins in yeast." Id.

The Invitrogen catalog is cited for teaching high copy number vectors for expression of proteins in P. pastoris, wherein the vectors include the inducible AOX1 promoter, a poly cloning site sequence, the alpha-factor signal sequence. See id. at 4-5.

The Answer asserts that:

> One of ordinary skill in the art would have been motivated to produce the claimed method and vectors and host cell because Horwitz [ ] teach[es] recombinant production of proteins, specifically, an antibody in S. cerevisiae in general with selection, screening, and purification and testing antigen binding. In addition,

> one of ordinary skill in the art would have been motivated to produce the claimed method and vectors and host cell in P. pastoris because Cregg [ ] teach[es] production of heterologous proteins in P. pastoris overcomes the problems associated with producing commercially useful levels of proteins in S. cerevisiae (see page 33, introduction) and the P. pastoris is ideally suited for the production of many heterologous proteins due to the fact that (1) a detailed understanding of the growth characteristics of the organism in high-density fermentors is known, (2) the ability to place foreign DNA into the genome in a precisely controlled manner, and (3) promoters are tightly regulated and efficiently transcribed to produce proteins at high levels (See page 40). In addition, one of ordinary skill in the art would have been motivated to produce the claimed method and vectors and host cells because the Invitrogen Catalog teach a Pichia expression vector called pPICZ which is based on homologous recombination comprising; several restriction sites for cloning of recombinant proteins, a promoter (AOX1), termination sequences, selectable markers (zeocin), and alpha-factor secretion signal for expression in P. pastoris of antibodies and the vector is designed for production of proteins as high as grams per liter (see pages 14-15 and 18). Moreover, one of ordinary skill in the art would have been motivated to produce the claimed method and vectors and host cells because Robinson [ ] teach[es] production in yeast of chimeric or humanized antibodies using a vector with both a light chain and a heavy chain linked to promoters and terminators in a single plasmid and the vectors can further comprise yeast leader sequences for antibody secretion (see columns 15-16).

Id. at 5-6.

The rejection also contends that there is a reasonable expectation of success because Horwitz teaches the production of antibodies in yeast that have the ability to bind to antigen. See id.at 6. Moreover, Cregg teaches that the production of heterologous proteins in P. pastoris may be easily scaled up, and the Invitrogen Catalog teaches that "the expression vector and P. pastoris makes

'an ideal tool for laboratory research as well as industrial applications.'" Id. at 6-7.

"[T]he Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. '[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references.'" In re Fritch, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (citation omitted). An adequate showing of motivation to combine requires "evidence that 'a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed.'" Ecolochem, Inc. v. Southern Calif. Edison Co., 227 F.3d 1361, 1375, 56 USPQ2d 1065, 1076 (Fed. Cir. 2000). In our view, the examiner has met that burden, and the rejection is affirmed.

Appellants argue that there is "no motivation to make and use dual expression cassettes for antibody production in Pichia." Appeal Brief, page 8. According to appellants, Robinson, which was relied upon for its teaching of a dual cassette vector, does not suggest the use of the dual cassette vector in Pichia, and does not even mention Pichia. See id. at 8-9.

Appellants contend that "the Office has erroneously interpreted the word 'yeast' as used in the context of Robinson to mean a genus of microorganisms that encompassed *Pichia*." Id. at 9. Relying on the declaration of Dr. James

Trager, appellants assert that yeast as used in Robinson refers only to *S. cerevisiae*, and not to *Pichia*, and there is no teaching, suggestion or motivation to use dual expression cassette vectors in *Pichia*. See id. at 9-10.

We agree with appellants that Robinson does not teach the use of a dual cassette vector to produce antibodies in *Pichia*, and that the reference does not mention the use of *Pichia* for the production of antibodies. Appellants, however, are arguing the Robinson reference separately, whereas the rejection was made over a combination of references. Assuming arguendo that the Robinson reference refers only to *S. cerevisiae* when it uses the term yeast,[3] the combination of references relied upon by the rejection render the method of claim 36 obvious.

Horwitz teaches a method for the functional secretion of an antibody from the yeast, *S. cerevisaiae*, wherein the gene encoding the light chain is placed on one expression plasmid, and the gene encoding the heavy chain is placed on a second expression plasmid. See Horwitz, abstract, and page 8679, column 2. Robinson teaches and exemplifies the same antibody expression system as

[3] We note that appellants assert that the term "yeast," if not referring to only *S. cerevisaiae*, "refers to a genus of fungi that encompasses over 25,000 species from the following families *Saccharomyces*, *Pichia*, *Candida*, *Schizosaccharomyces*, *Neurospora*, and others." Appeal Brief, page 11. The term yeast may refer to, however, yeast are that are known expression systems. As that argument has not been made, we do not rely on that interpretation, but merely bring it to the examiner's and appellants' attention to consider in any continued prosecution.

taught by Horwitz, see Robinson, col. 16, lines 21-34 and col. 44, Example 5, but also teaches that an alternative approach for simultaneously expressing both light and heavy chains in yeast is to attach the light and heavy chains to a yeast promoter and a terminator sequence and place both expression cassettes on the same plasmid, see id. at col. 16, lines 15-20. The combination thus teaches that one can use a single vector, dual cassette expression system, to express functional immunoglobulin in the yeast *S. cerevisiae*.

The rejection relies upon Cregg and the Invitrogen catalog for their teaching of the use of the yeast, *Pichia pastoris*, for the production of heterologous proteins. As noted by the rejection, see Examiner's Answer, page 5, Cregg teaches that problems exist with scaling up the production of heterologous proteins in yeast, and teaches that in light of those problems, a second-generation yeast expression system, *Pichia pastoris*, has been developed as a host system for the efficient, large-scale production of heterologous proteins. We therefore find it would have been obvious to the ordinary artisan at the time of invention to substitute the *Pichia* expression system in the *S. cerevisiae* dual cassette, single vector expression system as taught by Robinson and Horwitz because of the advantages of the *Pichia* expression system as taught by Cregg and the Invitrogen catalog.

Appellants argue further that the art provides no reasonable expectation of success, and that the art in fact teaches away from the claimed invention. See Appeal Brief, page 12. First, appellants rely on Pinnell for teaching that

"'The size of the protein to be expressed may also be limiting because to our knowledge, there are no reports of proteins greater than 117 kDa being expressed in *P. pastoris*.'" Id. at 12 (emphasis in original) (quoting Pinnell, page 601). Appellants assert that statement teaches away from the claimed invention, as antibodies are generally larger than 117 kDa. See id.

Second, appellants rely upon Holliger for teaching that: "'Because bicistronic expression works only poorly in *Pichia* (unlike *E. coli*), it is preferable to use single-chain Ab formats. Two chain Ab formats require that the two chains be cloned and transformed separately.'" Appeal Brief, page 13 (emphasis in original) (quoting Hollinger, page 351). Dr. Trager also reviewed Pinnell and Hollinger, and appellants cite paragraph 22 of his declaration to support their proposition that the references would lead the ordinary artisan away from the claimed invention.

Again, we do not find appellants arguments to be convincing. With respect to the statement of Pinnell that "[t]he size of the protein to be expressed may also be limiting because to our knowledge, there are no reports of proteins greater than 117 kDa being expressed in *P. pastoris*," as noted by the examiner, the Invitrogen catalog teaches the expression of a wide variety of proteins that have been expressed in *Pichia*, such as GP-120, see Examiner's Answer, page 9, which appellants do not dispute is larger than 117 kDa.

With respect to the statement of Hollinger, appellants cannot rely on post-filing art references to show what one skilled in the art would know at the

time of filing. The state of the art at the filing date of the application is used to determine whether a particular disclosure is enabling as of the filing date. Publications dated after the filing date providing information publicly first disclosed after the filing date generally cannot be used to show what was known at the time of filing. See In re Gunn, 537 F.2d 1123, 1128, 190 USPQ 402, 405 (CCPA 1976). We acknowledge that Hollinger is a review article, but we could find no citation in Hollinger to an earlier filed publication that it was known at the time of filing of the instant application that bicistronic expression works only poorly in *Pichia* (unlike *E. coli*), and that two chain Ab formats require that the two chains be cloned and transformed separately.

Finally, we acknowledge Dr. Trager's statement in paragraph 22 of his declaration, but as his statements are based in part on the above statements in the Pinnell and Hollinger references, which we do not find teach away from the claimed invention for the reasons set forth above, we also find paragraph 22 of the declaration not to be convincing on the issue of obviousness. Moreover, all that is required is a reasonable expectation of success, not absolute predictability of success. See In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988). Given the teachings of the Invitrogen Catalog that a wide variety of proteins have been expressed using the *Pichia* expression system, see Table 1, one of ordinary skill would have had a reasonable expectation of success of using a dual cassette, single vector expression system to express a functional immunoglobulin protein in the *Pichia* expression system.

Citing paragraph 15 of the Trager declaration, appellants also argue that "protein expression is unpredictable, and successful heterologous protein expression in *S. cerevisiae* does not predict successful heterologous protein expression in *Pichia*." Appeal Brief, page 13. Moreover, according to the declarant, "[e]ven if a reference was cited that actually showed a working method for the expression of functional antibodies in *S. cerevisiae* using a dual expression cassette vector, it is my unequivocal opinion that a Skilled Person would have no reasonable expectation of success in practicing such a method in *Pichia*." Id. at 14 (quoting Trager Declaration, ¶16). Appellants thus conclude that a person of ordinary skill in the art must make three leaps from the disclosure of Robinson—the first being that yeast as used in Robinson means something other than *S. cerevisiae*, the second being that evidence of a single expression cassette vector for antibody production in *S. cerevisaiae* is predictive of success using a dual cassette system, and the third being that expression in *S. cerevisaiae* is predictive of success in *Pichia*—and the Pinnell and the Hollinger references, as well as the Trager declaration, "provides ample evidence that none of these leaps are trivial, and that the ordinary skilled artisan would not make these leaps." Appeal Brief, page 14.

We do not find appellants' arguments convincing for the same reasons as set forth supra. As noted above, obviousness only requires a reasonable expectation of success, not an absolute predictability. If we were to accept declarant's arguments that protein expression is unpredictable, and successful

heterologous protein expression in *S. cerevisiae* does not predict successful heterologous protein expression in *Pichia*, an obviousness rejection would never be appropriate anytime one changed to a new expression system. With respect to the statements in paragraph 17 of the Trager declaration, the problems recited, i.e. intra-molecular recombination, transcriptional interference and translational interference, relate to the expression in any expression system, and are not specific to *Pichia*.

Claims 36-39 and 41-50 stand rejected under 35 U.S.C. § 103(a) as being obvious over the previous combination of references as further combined with Vanderlaan. As appellants merely argue that Vanderlaan does not remedy the deficiencies of the previous rejection, this rejection is affirmed as well.

CONCLUSION

As we find that the rejections under 35 U.S.C. § 103(a) set forth a prima facie case of obviousness that has not been adequately rebutted by appellants, we affirm.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.13(a).

AFFIRMED

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